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13. ABSTRACT *(Maximum 200 words)*

Three main strategies in the application of CRDS to liquids have been investigated. In the direct liquid approach, an enclosed cavity is entirely filled with liquid for the purpose of quantifying low concentrations of solutes in a bulk solution and monitoring reactions in solution. A picomolar detection limit for a strongly absorbing solute in acetonitrile has been demonstrated, and interesting kinetic behavior has been observed at low concentrations. In the Brewster's angle flow cell implementation, a specially designed sample cell is placed within an optical cavity and used to detect low concentration species in small volume aqueous samples either statically or in a flow. It has been successfully coupled to liquid chromatography and promises to surpass commercial UV-Vis detectors in sensitivity. In evanescent wave CRDS, a prism is placed within the optical cavity such that light undergoes total internal reflection within the prism, and the resulting evanescent wave is employed to probe optical absorption at the glass-water interface. This technique has been used to investigate cation adsorption to the interface and may have applications as an ultrasensitive detector of anionic surfactants.

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Application of Cavity Ring-Down Spectroscopy to Liquid Samples
Final Technical Report

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LONG-TERM GOALS

The goal of this project was to investigate the sensitivity and applicability of cavity ring-down spectroscopy (CRDS) as a tool for the measurement of trace quantities of chemical and biological solutes in solution. Possible implementations of a field instrument and extensions to directly target chemical and biological contaminants of interest to the Office of Naval Research also have been considered.

OBJECTIVES

The objectives of the research were to identify and implement CRDS techniques for the detection and quantitation of chemical species in liquid samples. This investigation has begun to assess the sensitivity and applications of each implementation and to suggest possible improvements.

APPROACH

Three main strategies in the application of CRDS to liquids have been investigated. Elena S. F. Berman, Davida J. Ankeny Brown, and Alexander J. Hallock have filled an enclosed cavity entirely with liquid to quantify low concentrations of solutes in a bulk solution and to monitor reactions in solution. Kate L. Snyder has designed and implemented a Brewster's angle flow cell that when placed within an optical cavity is used to detect low concentration species in small volume aqueous samples either statically or in a flow. Theresa E. Hannon, Fuping Li, and Andrew M. Shaw have placed a prism within the optical cavity such that light undergoes total internal reflection within the prism, and the resulting evanescent wave was employed to probe optical absorption at the glass-water interface.

Alexander Hallock was a Ph.D.student in the Zare group through October 2002, and is now a postdoctoral research associate at Columbia University. Andrew Shaw was a postdoctoral research associate in the Zare group through September 2001, and is now a faculty member at the University of Exeter in the UK. All other researchers mentioned here are currently Ph.D. students in the Zare group.

WORK COMPLETED

a) Direct Liquid CRDS (DL-CRDS). A picomolar detection limit for a strongly absorbing solute in acetonitrile has been demonstrated. Efforts have begun to miniaturize the

apparatus for field use, and reactions in solution have been monitored using this technique.

b) Brewster's Angle Flow Cell CRDS. This project has successfully coupled an analytical separation technique (high performance liquid chromatography) with Brewster's angle CRDS to achieve a sensitivity comparable to, if not better than, a high-quality commercial UV-Vis detection instrument. These results were obtained with a simple pulsed CRDS system, and we expect to improve our detection limit by 1-2 orders of magnitude by switching to a single-mode cw laser source.

c) Evanescent Wave CRDS (EW-CRDS). An investigation of cation competition at the glass-water interface has been completed. Improvements to the system have been made in order to increase the sensitivity, and studies have begun to evaluate the effectiveness of EW-CRDS for the ultrasensitive detection of anionic surfactants in aqueous solution.

RESULTS

a) Direct Liquid CRDS (DL-CRDS). The simplest application of CRDS to liquids involves completely filling the optical cavity with the sample. This approach minimizes losses due to the number of interfaces but has the effect of reducing the ring-down time due to the absorption and scattering of light by the solvent. Using acetonitrile as the solvent results in a maximum ring-down time of 400 ns in a cavity that is approximately 22 cm. When the sample is added, the losses in the cavity increase, and a change in the ring-down time is observed. The sensitivity of the technique is determined by the minimum detectable change in the ring-down time and the losses due to the solvent. In the visible region, we have shown that the technique is capable of measuring changes in the absorption coefficient from 10^{-3} down to 10^{-6} cm^{-1}.

Recently, we have begun using DL-CRDS to observe the kinetics of chemical reactions. Specifically, we have been interested in the reduction of methylene blue (MB^+) by ascorbic acid to leucomethylene blue. In the literature,[1] this reaction is known to proceed with kinetics that are first order in MB^+ with water as the solvent. In our DL-CRDS apparatus, we have been studying this reaction in acetonitrile at nanomolar concentrations of MB^+. In this nonpolar solvent and at such low concentrations, we clearly observe more complex kinetics, shown in Figure 1. We observe a loss of MB^+ which is second order in the concentration of MB^+, followed at long times by an equilibration which appears to result from a back reaction with dissolved oxygen. We propose a radical mechanism to explain the second-order behavior; more studies are planned to support this mechanism.

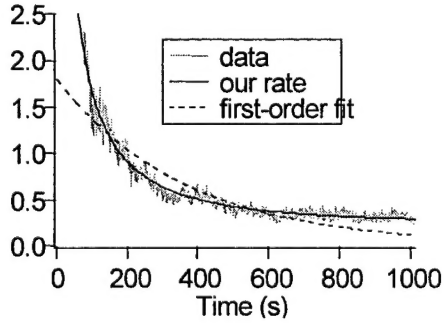

Figure 1. The reaction of methylene blue with ascorbic acid.

In addition, significant changes to the instrumentation are underway (Figure 2), all of which bring us closer to the intertwined goals of enhanced sensitivity, miniaturization, and field instrument production. Nearly every component of the apparatus has been or will be modified in some way, including the laser light source, coupling of the light source to the cavity, the photomultiplier tube, the oscilloscope, and the computer.

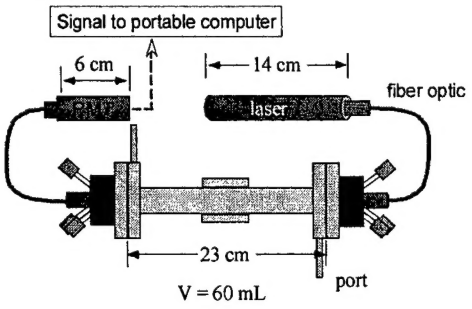

Figure 2. Proposed setup of the miniaturized system.

b) Brewster's Angle Flow Cell CRDS. In this system, the non-absorption optical losses in the ring-down cavity are reduced by incorporating a liquid flow cell that has been designed to direct the light through each interface at Brewster's angle. With the correct polarization of light, the reflections will be minimized at each surface, allowing the light to pass through the cell hundreds of times. Ring-down time constants of 2.5 μs are obtained with pure water in the 300-μm optical pathlength cell in a 1 m cavity. In the static measurement case, concentrations of quinalizarin, an anthraquinone, ranged from 30 nM to 30 μM with an R^2 value of 0.9998 – three orders of magnitude linear dynamic range! This system was successfully coupled to high performance liquid chromatography (HPLC). Figure 3 depicts the experimental setup of HPLC coupled to CRDS.

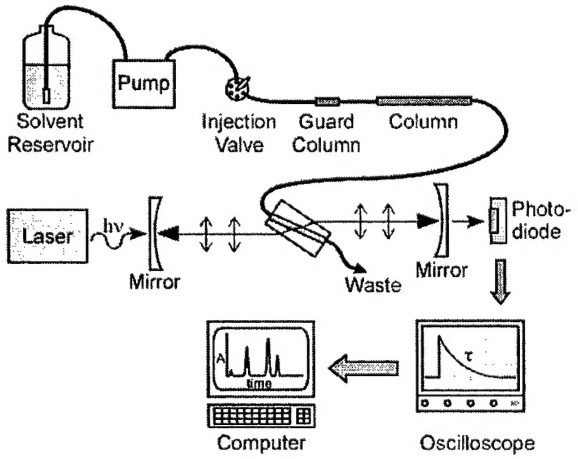

Figure 3. Experimental setup of the HPLC-CRDS system.

To test this system, five molecules in the anthraquinone family were injected and analyzed by both our HPLC-CRDS system and a commercial UV-Vis detection instrument. Figure 4 is the separation as detected by CRDS and Figure 5 is the separation as detected by UV-Vis. The concentrations of the species in Figure 5 are lowered to account for the longer pathlength of the UV-Vis sample cell. The two figures are on the same y-axis scale of absolute absorbance units.

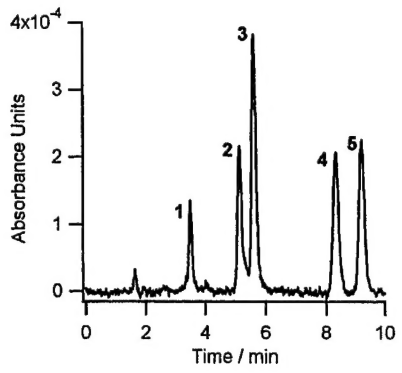

Figure 4. An HPLC separation of 10 μM of (1) alizarin, (2) purpurin, (3) quinalizarin, (4) emodin, and (5) quinizarin using CRDS detection in a 300-μm-pathlength cell. The peak shortly before two minutes is the solvent front.

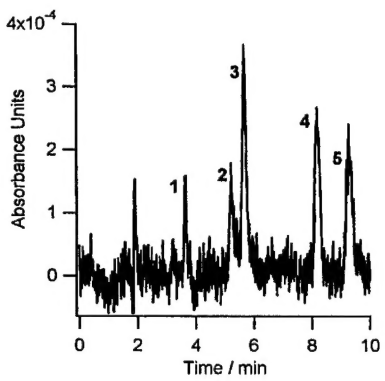

Figure 5. An HPLC separation of 0.3 μM of (1) alizarin, (2) purpurin, (3) quinalizarin, (4) emodin, and (5) quinizarin using standard UV-Vis detection in a 10-mm-pathlength cell. The peak shortly before two minutes is the solvent front.

As can be seen from these chromatograms, CRDS utilizing a Brewster's angle flow cell is quite powerful and worth investigating further.

c) Evanescent Wave CRDS (EW-CRDS). Placing a total internal reflection (TIR) element such as a Dove prism or a triangular prism within the optical cavity provides a means to monitor absorption in a lower-refractive-index condensed phase near the prism surface.[2] The evanescent wave formed with TIR extends only a few hundred nanometers into the lower-index chemical medium and decays exponentially with distance from the interface. These factors make the technique suitable for the detection of species that preferentially adsorb to the interface or for the study of small changes in interfacial adsorption. EW-CRDS has been extended to the study of liquid-phase adsorption processes, employing a flow cell secured to the prism. We routinely measure changes in absorbance on the order of 10^{-4}, though experiments indicate that it may be possible to measure absorbances one to two orders of magnitude lower than this with the current pulsed laser system.

In a recent study, we monitored the attraction of a cationic dye, crystal violet (CV^+), to the glass surface. The interfacial absorbance by CV^+ at 630 nm was measured as a function of pH and salt content in the aqueous dye solution (Figure 6).

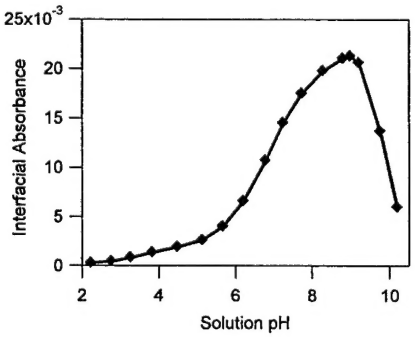

Figure 6. Competition between CV^+ and K^+ at the glass-water interface monitored by EW-CRDS. The bulk solution is 66.5 μM crystal violet, 0.25 M KCl.

Since the completion of that project, we have designed and implemented a new triangular prism to minimize optical losses within the cavity. Currently, we are developing a sensor for anionic surfactants in aqueous solution based upon a variation of the "methylene blue active substances" method.[3,4] Following this method, the surfactant is allowed to complex with methylene blue, and then the interfacial absorbance is measured as the complex attracts to a hydrophobic coating at the TIR surface. We also have begun to investigate molecular orientation at the TIR interface by comparing the relative interfacial absorbances of s- and p-polarized incident light.

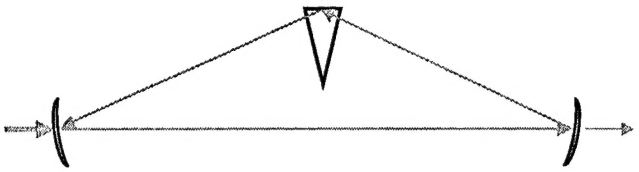

Figure 7. Experimental setup containing new prism.

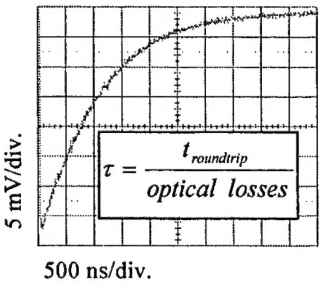

Figure 8. Representative decay trace with new setup. Decay constant $\tau \sim 975$ ns.

IMPACT/APPLICATIONS

Three different methods have been employed to apply CRDS to the liquid phase. All of these methods have been shown to possess enhanced sensitivity over more conventional absorption spectroscopies. Specifically, direct liquid CRDS is applicable to monitoring fast reactions in solution. Brewster's angle flow cell CRDS is suitable for the detection of small-volume analytes following a separation method. EW-CRDS is effective for interface-specific studies.

Several of the recent advances in this project are enabling us to work towards the development of a portable field instrument for the ultrasensitive detection of harmful contaminants in liquids. For example, combining a diode laser light source with a Brewster's angle flow cell cavity following HPLC could provide a portable device for the separation and detection of trace contaminants in low-volume, low-concentration samples. EW-CRDS is applicable for field studies of solutes that can be preconcentrated at a solid-liquid interface. Direct liquid CRDS is likely to be particularly useful for harsh environmental conditions in which a more mechanically robust cavity is needed.

TRANSITIONS

These liquid-phase CRDS techniques have the potential to be used by analytical and physical chemists for numerous high-sensitivity absorption experiments.

RELATED PROJECTS

Dr. Heather Rypkema and Marion Martin in our laboratory have continued work on Bounce-by-Bounce CRDS. This newly developed technique expands the application of cavity ring-down spectroscopy to chemical processes in which the absorptive properties of the sample

are changing on a timescale comparable with the ring-down time. By resolving the signal attenuation pulse by pulse rather than as a decay envelope it becomes possible conclusively to identify nonexponential behavior as well as discriminate between exponential and compound exponential behavior.

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PROVISIONAL PATENT APPLICATIONS

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